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# THE ANTIGENIC PROPERTIES OF GELATIN

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Notwithstanding the large amount of work given to the subject, the exact character of that group of substances called "antigens" remains obscure. Whether or not chemical analysis will be able to determine this function as a property of any certain chemical group or groups is questionable. Indications seem to point to the idea that physical factors[1]—the molecular size of the substance, the degree of dispersion, the arrangement of certain groups within the molecule—play a part, as well as the strictly chemical composition. As Wells[1] has said, Whether the chemical differences that determine specificity are of a quantitative nature which can be disclosed by analytic means or whether they are dependent on spatial relationship of the amino-acid radicals, as suggested by Pick,[2] remains to be determined."

The definition of an "antigen" at present is based, not on the chemical composition of the substances but on the peculiar biologic reactions, which make themselves manifest in certain definite ways. It is only as it is capable of fulfilling the conditions of these reactions, that any substance is called an antigen.

Certain general facts, however, are at present fairly well established, as regards antigens. They are proteins, relatively complex in character, with relatively large molecules. The antigenic properties seem to parallel the molecular size and the chemical complexity. The fact cited by Pick that certain types of proteins call forth lytic reactions, while other types call forth agglutination and precipitin reactions, indicates that the function must be dependent on different groups of the molecule. The molecule must evidently possess certain chemical groups in order to call forth certain types of antibodies.

Obermeier, Pick, Vaughan, and others have made the observation that a protein to possess antigenic functions must contain amino-acids

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[1] Chemical Pathology, 191, p. 187.

[2] Pick: Biochemie der Antigene, u. s. w. Kolle und Wassermann's Handbuch der Pathogenen Mikroorganismen, 1913, 1, p. 685.

rich in aromatic radicals. According to them, proteins containing none of these radicals serve poorly, if at all, as antigens. This is of peculiar interest in relation to the antigenic properties of gelatin.

Attempts to obtain some knowledge as regards the chemical basis of antigens have been many and varied. They have been conducted by the preparation of definite types of chemical compounds in as pure a state as possible and then studying the antigenic properties of the compounds. Thus, Wells and Osborne[3] have prepared and tested many pure vegetable proteins; Elsesser[4] likewise tested various vegetable proteins for the production of proteolytic ferments; Wells[6] studied various nucleo-proteins; Elliott[7] studied glycoproteins from various sources; Schmidt[8] prepared certain compound proteins, e. g., protamine-edestinate, and studied their antigenic properties; Dale and Hartley[9] tested the antigenic properties of the fractions of horse serum. These are only a few examples indicative of the large amount of work that has been done along this line. An extensive review of these studies is given by Pick.

In view of these studies and particularly because of the peculiar chemical composition of gelatin, the sugg[illegible] was made that i[illegible]erties be studied.

Gelatin is a rather peculiar protein compound, th[illegible] composition of which is not known. In fact, there i[illegible] the term "gelatin" as now used represents a chemical entity. Chemical analyses of gelatin derived from different sources or prepared by different methods, vary considerably.[10] They all agree, however, in the general result, that gelatins are rich in glycocoll, proline, and arginine, but poor or entirely deficient in the amino-acids that contain the aromatic radicals. The study, therefore, of its antigenic properties might be expected to yield some information regarding the relation of the aromatic radicals to antigenic function.

In the literature one can find only a few references to gelatin as an antigen. The first experiments found recorded in the literature are those of Wells,[11] who was unable to secure anaphylactic reactions with gelatin in guinea-pigs, which lack of anaphylactogenic property he ascribed to the deficiency of aromatic radicals in gelatin. Arthus[12] reports that he was able to sensitize rabbits with gelatin, by intravenous injections. After 5 or 6 injections, he observed lack of absorption of injected material and abscess formation, such as characterizes the reaction of local anaphylaxis (Arthus' phenomenon). Abderhalden[13] reports that the repeated injections of dogs with gelatin are accompanied by the formation of proteolytic ferments, quantitatively specific for gelatin, which may be demonstrated by optical methods. Ascoli and Izar,[14] and Izar[15] report changes in the blood of rabbits repeatedly injected with gelatin which are manifested by changes in the viscosity of the antigen-antibody mixture (meiostagmin reaction). Pick[1] states that gelatin is not capable of stimulating to the production of antibodies. Wells,[16] in discussing anaphylaxis, says, "So far as known, antigens are always protein, and with the *exception* of *gelatin* and a few others, all proteins will produce sensitization in susceptible animals." Pabes and Regazzi[17] sought to produce anaphylactic reactions in rabbits by repeated injections with gelatin, both normal and hydrolyzed, with negative results. They suggest that the cause of the failure is in the character of the gelatin molecule, which [illegible] course, means nothing, [illegible] the light of our present ignorance of the [illegible] of gelatin.

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[3] Jour. Infect. Dis., 1911, 8, p. 106.

[4] Ibid., 1916, 19, p. 655.

[6] Jour. Biol. Chem., 1916, 28, p. 1.
Ztschr. f. Immunitätsf., O., 1913, 19, p. 598.

[7] Jour. Infect. Dis., 1914, 15, p. 501.

[8] Univ. of Calif. Pub. Path., 1916, 2, p. 157.

[9] Biochem. Jour., 1916, 10, p. 408.

[10] Mathews, A. P.: Physiological Chemistery, 1916, Ed. 2.
Greifenhagen, König u. A. School-Biochem. Ztschr., 1911, 35, p. 217.
Sadikoff: Ztschr. f. Phys. Chemie, 1904, 41, p. 15.
Sadikoff: Ztschr. f. Phys. Chemie, 1903, 39, p. 411.
Plimer: Practical Organic and Bio-Chemistry, 1915, p. 453.
Mörner: Ztschr. f. Phys. Chemie, 1899, 28, p. 471.
Sadikoff: Ztschr. f. Phys. Chemie, 1903, 39, p. 396.
Cohnheim: Chemie der Eweisskörper, 1904, p. 285.
Hoppe-Seyler: Handbuch der Phys. u. Path. Chemischen Analyse, 1909, p. 449.
Allen: Commercial Organic Analysis, 1913, Ed. 4, 8, p. 587.

[11] Jour. Infect. Dis., 1908, 8, p. 88.

In view of the conflicting results reported, of the lack of detailed account [illegible] the experimental methods, and also because in many instances the attempt was made to show only one kind of antibody, it was thought that a more detailed immunological study of gelatin would be worth while.

For this purpose, two kinds of gelatin were used. One was prepared by purification of the best grade of laboratory gelatin obtainable —the French "Gold Leaf." The other type was prepared from the tracheal rings of the beef, giving the product generally called "Cartilage Gelatin," or "Chondrin." The latter was used in a few instances only, serving as specificity control. It has been made the subject of a special investigation, as yet unpublished.[18] Unless specifically noted, the first type of gelatin is that which is referred to in this article.

Since very small traces of proteins are capable of acting as antigens, great care was exercised to avoid accidental contamination with other proteins. The glassware, apparatus, syringes, were all soaked in a strong bichromate-sulphuric acid cleaning mixture for 24–48 hours and then thoroughly washed in cold and hot distilled water. Such apparatus was then used for the gelatin work only. In view of the constant results obtained throughout, it is evident that no foreign proteins, at least capable of acting as antigens, were present.

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[12] Arch. Internat. de physiol., 1908, 7, p. 493.

[13] Ztschr. f. Phys. Chemie, 1910, 64, p. 100.

[14] München. med. Wchnschr., 1910, 57, pp. 62, 403, 954, 2129.

[15] Ztschr. f. Immunitätsf., O., 19, 7, p. 189.

[16] Chemical Pathology, 1914, p. 179.

[17] R. acct. Fisiocril, 221, p. 557.

[18] Peeples: Master's Thesis, Ohio State Univ., 1917.

## PREPARATION OF GELATIN

(1) *"Gold Leaf" Gelatin.*—Three separate unbroken samples of this product were obtained. An aliquot part was taken from each sample and the mixture used. The mixture was washed in cold water for 24 hours at refrigerator temperature. It was then dried, and washed in two changes of absolute alcohol for 24 hours. This was followed by washing with ether, two changes, for 24 hours and dried. After drying, the gelatin was dissolved in a minimum amount of warm water. Care was exercised not to have the water hotter than necessary for dissolving, thus attempting to avoid high temperatures which might influence the antigenic properties of the substance. The dissolved gelatin was precipitated by pouring the solution, with constant stirring, into 10 volumes of 95-98% ethyl alcohol. A heavy, white, very tenacious precipitate resulted. This precipitate was allowed to remain in contact with the alcohol for 24 hours or more, when the alcohol was decanted off, and the precipitate redissolved in a minimal amount of warm water. This dissolved the gelatin but left the other alcohol-precipitated material undissolved. This solution was passed through filter paper and reprecipitated into 10 volumes of fresh ethyl alcohol, in which it was again allowed to remain 24 hours. This process of precipitation with alcohol, re-solution in warm water, and filtration, was repeated 4 times. The product following final precipitation was cut up into as fine shreds as possible and washed 24 hours in absolute alcohol in the cold, followed by boiling in absolute alcohol on a water bath, for 20-30 minutes. The alcohol was removed by decantation and the product washed in ether for 24 hours with frequent stirring. This application of ether was repeated, the final ether allowed to evaporate, and the material dried over sulphuric acid in a vacuum desiccator.

The product obtained is hard and horny in consistency—golden to grayish-yellow in color. It is readily soluble in warm water and gels on cooling to 20 C., even in dilutions of 1%.

Application of the precipitation, solubility, and colorimetric tests for gelatin as indicated by Mörner, Sadikoff, Hoppe-Seyler, and Allen gave results which were in conformity to those characteristic of gelatin. According to Berriar[36] gelatin can be separated from other proteins and quantitatively determined by adding to the gelatin solution a mixture of 1 part of picric acid (sat. aq. sol.) plus 4 parts of absolute alcohol At 80 C. all other proteins are precipitated while the gelatin is held in solution. On evaporation of the alcohol following filtration, the gelatin is precipitated quantitatively as cooling progresses. The application of this method to the product obtained gave no indication of any proteins present other than gelatin.

(2) *Cartilage Gelatin (Chondrin).*—For details regarding the preparation of this product, I refer to Allen's Chemical Commercial Organic Analysis. In outline, the method consists in digestion of the tracheal rings of the beef, after removal of all connective tissue, with dilute lime water, under steam pressure, precipitation of the filtered digest with 10-15 volumes of absolute alcohol. Redissolve in water; reprecipitate with acetic acid; filter, neutralize with sodium carbonate; reprecipitate in absolute alcohol, after which the treatment is the same as for the other type of gelatin. The resulting product is a golden brown substance, finely granular, easily soluble in water. It gives the tests regularly ascribed to chondrin.

### TOXICITY OF GELATIN

Investigators[19] who have injected animals report that normal gelatin possesses considerable toxicity and thus they experienced considerable difficulty in a series of injections into animals. While no definite attempt was made to determine the lethal dose of the gelatin in this work, the results of a large series of injections would indicate that the toxicity must be relatively low, and that animals tolerate large quantities without any marked permanent ill effects. For example, rabbits were repeatedly injected intravenously, with amounts varying from 0.2-0.4 gm. on each of 3 successive days, without any fatal results. Such injections were, however, accompanied by some loss of weight and by stupor. Intravenous injections of amounts over 0.5 gm. into rabbits resulted fatally, in the few tests made. Neither was there any evidence of any cumulative action. As noted later, many of the rabbits received 10-25 injections, intravenously or intraperitoneally, over a period of 5-10 months without any indications of intoxication.

Guinea-pigs withstood intravenous injections of 0.1 gm. without toxic results being evident.

## TYPES OF ANTIBODY REACTIONS STUDIED

The following types of antibody reactions were studied with serum from guinea-pigs, rabbits, and dogs, injected in various ways with gelatin: (a) anaphylaxis; (b) precipitins; (c) production of specific proteolytic ferments; (d) complement binding; (e) meiostagmin reaction.

### THE ANAPHYLAXIS REACTION

With the exception of Arthus,[12] who reports positive results in rabbits, the literature reveals that other investigators have obtained negative results in attempting to demonstrate that gelatin is an anaphylactogen. Only very meager reports, however, are obtainable, most authors simply stating that gelatin gave negative results, or that no general symptoms were observed. In carrying out anaphylactic experiments in this investigation, rabbits, guinea-pigs, and dogs were used, and conclusions as regards results were based on (a) general symptoms, (b) variations in temperature following second injection, (c) the character of the blood pressure (in dogs), (d) changes in coagulation time of blood, and (e) necropsy findings.

### EXPERIMENTS ON RABBITS

Young rabbits, 700-1,100 gm., were used. The gelatin was dissolved in sterile physiologic salt solution, generally diluted in such a way that 1 c c contained the desired amount for injection. The sensitizing injections were all made intraperitoneally, in doses from 0.04-0.1 gm. The intoxicating dose used was always much larger, being from 5-10 times the sensitizing dose, and injected either intravenously or intraperitoneally. The interval elapsing between the first and second injection was about 16 days. When a third injection was given, this was 10-12 days subsequent to the second. All the usual precautions of cleanliness of apparatus were observed to avoid any possibility of sensitization with other alien proteins.

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[19] Aronson: Personal communication to Dr. H. G. Wells, 1916.
Buglia: Biochem. Ztschr., 1908, 23, p. 215.

Results are recorded in accordance with the method suggested by Wells.[20] The same significance is attached to the terms throughout all the anaphylactic work.

TABLE 1

ANAPHYLAXIS EXPERIMENTS WITH GELATIN ON YOUNG RABBITS

| Rabbit | Weight, gm. | Sensitizing Injection | | Interval, Days | Second Injection | | Symptoms | Interval, Days | Third Injection | | Symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Amount, gm. | Mode | | Amount, gm. | Mode | | | Amount, gm. | Mode | |
| 1 | 785 | 0.1 | INTRAPERITONEAL | 16 | 0.8 | Intra-peri-toneal | Negative. Slight restlessness | 12 | 0.8 | Intra-peri-toneal | Negative |
| 2 | 785 | 0.04 | | 17 | 0.2 | INTRAVENOUS | Negative | 12 | 0.2 | INTRAVENOUS | Same as after injection No. 2 |
| 3 | 775 | 0.04 | | 16 | 0.2 | | Doubtful. Normal in 4 hours | 12 | 0.2 | | Same as after injection No. 2 |
| 4 | 952 | 0.04 | | 17 | 0.4 | | Doubtful. Slight irritation. Normal in 1½ hours | 10 | 0.4 | | No change |
| 5 | 870 | 0.1 | | 16 | 0.8 | INTRA-PERITONEAL | Negative | 10 | 0.8 | INTRA-PERITONEAL | No change |
| 6 | 1,093 | 0.1 | | 16 | 1.0 | | Doubtful. Temperature fall of 1.8 C. | 10 | 1.0 | | Negative |
| 7 | 1,000 | ... | .. | .. | 1.0 | | Doubtful. Restless and stupid | 12 | 1.0 | | Doubtful |
| 8 | 900 | 0.05 c c ox serum | Intra-peri-toneal | 16 | 1.5 c c ox serum | Intra-venous | Severe. Death in 35 minutes | .. | | | |

No. 7—Control rabbit.
No. 8—Control rabbit.

"1. 'Doubtful' Symptoms: The animal scratches itself a few times and perhaps seems a little uneasy, or a trifle ill; the temperature remains normal or falls not more than 1-1.5 C. Such a reaction may occasionally be observed after injections of foreign serum into nonsensitized animals, and is not to be regarded as of any significance.

"2. 'Slight' Symptoms: The animal does not become seriously ill but scratches itself vigorously, is either restless or very somnolent, or both alternately, hair roughened, eyes usually lacrimate and partially closed. The temperature falls 1-1.5 C. Such a reaction is believed to indicate, usually if not always, a real sensitization and a true anaphylactic intoxication, but it may possibly, sometimes, though rarely, occur from the injection of foreign proteins without sensitization.

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[20] Jour. Infect. Dis., 1911, 8, p. 88.

"3. 'Moderate' Symptoms: More seriously ill than under 2. Usually the hair becomes rough, with respiratory disturbances and marked lacrimation, fall of temperature from 1.5-3.5 C.; frequently the animal lies down and sometimes there is moderate coughing.

TABLE 2

Anaphylaxis Experiments with Gelatin on Young Guinea-Pigs. Series 1

| Guinea-pig | Weight, gm. | Sensitizing Injection | | Interval, Days | Second Injection | | Symptoms | Interval, Days | Third Injection | | Symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Amount, gm. | Mode | | Amount, gm. | Mode | | | Amount, gm. | Mode | |
| 1 | 283 | 0.05 | INTRAPERITONEAL | 18 | 0.3 | INTRAPERITONEAL | Doubtful. Slight restlestness and stupor. Normal in 6 hours* | .. | ...... | .. | |
| 2 | 253 | 0.05 | | 18 | 0.5 | | Negative† | .. | ...... | | |
| 3 | 357 | 0.05 | | 18 | 0.3 | | Negative | 10 | 0.3 | INTRAPERITONEAL | Negative |
| 4 | 426 | 0.02 | | 18 | 0.3 | | Doubtful | 10 | 0.3 | | Negative |
| 5 | 329 | 0.02 | | 18 | 0.3 | | Negative | 10 | 0.5 | | Negative (stupor) |
| 6 | 352 | 0.02 | | 18 | 0.5 | | Negative | 10 | 0.5 | | Negative |
| 7 | 365 | 0.005 | | 20 | 0.3 | | Doubtful | 10 | 0.5 | | Negative |
| 8 | 305 | 0.005 | | 20 | 0.05 | Intracardial | Doubtful | 10 | 0.5 | | Doubtful |
| 9 | 293 | 0.005 | | 20 | 0.05 | | Negative | .. | ...... | .. | |
| 10 | 300 | 0.05 | Intracardial | .. | ...... | .. | Doubtful | | | | |
| 11 | 350 | 0.3 | Intraperitoneal | | | | Negative | | | | |

* Died 3 days later. No anaphylactic lesions. Pneumonia.
† Died 2 days later. No lesions typical of anaphylaxis. Pneumonia and ascites.
No. 10—Control.
No. 11—Control.

"4. 'Severe' Symptoms: Same as under 3, but so much more marked as to threaten death, but followed by complete recovery in a few hours. In these animals the temperature usually falls 3-4 C. or even more."

It may be noted that in these anaphylactic experiments with gelatin, not much stress can be laid on the relation of stupor to anaphylaxis, since it was found to be a rather common occurrence even among animals receiving the first injection, as also among those animals that had received 5-10 injections. I believe it is a symptom peculiar to the gelatin, per se, and not related to the phenomenon of anaphylaxis.

Table 1 will serve to summarize the technic and results obtained in the attempt to demonstrate anaphylaxis in rabbits with gelatin.

The results of these experiments indicate that gelatin is not capable of sensitizing young rabbits against subsequent injections of the same material. The "doubtful" symptoms noted in Rabbits 3, 4, and 6 were very mild and no more severe than those observed in control Rabbit 7. The third injection was made for the purpose of seeing whether there was any evidence of the appearance of a refractory stage following the second injection—antianaphylaxis. As will be observed, there was no indication of any changes in the character of the reaction which might be attributed to antianaphylaxis. The rabbits were kept under observation for a period of 10 days following the last injection; no abnormal symptoms were noted.

TABLE 3

ANAPHYLAXIS EXPERIMENTS WITH GELATIN ON YOUNG GUINEA-PIGS. SERIES 2

| Guinea-pig | Weight, gm. | Sensitizing Injection | | Inter-val, Days | Second Injection | | Symptoms | Inter-val, Days | Third Injection | | Symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Amount, gm. | Mode | | Amount, gm. | Mode | | | Amount, gm. | Mode | |
| 1 | 300 | 0.01 | INTRAPERITONEAL | 20 | 0.075 | INTRAJUGULAR | Doubtful | 12 | 0.1 | INTRAPERITONEAL | Negative |
| 2 | 275 | 0.01 | | 20 | 0.10 | | Doubtful | 10 | 0.3 | | Doubtful |
| 3 | 290 | 0.01 | | 20 | 0.075 | | Negative | 10 | 0.3 | | Negative |
| 4 | 300 | 0.005 | | 20 | 0.10 | | Doubtful | 10 | 0.3 | | Similar to 2nd injection |
| 5 | 280 | 0.005 | | 20 | 0.075 | | Doubtful | 10 | 0.3 | | Doubtful |
| 6 | 275 | 0.005 | | 20 | 0.10 | | Doubtful | 10 | 0.2 | | Doubtful |
| 7 | 300 | 0.15 | Intra-jugular | .. | ...... | .. | Doubtful | .. | ...... | .. | |
| 8 | 360 | 0.15 | | .. | ...... | .. | Doubtful | .. | ...... | .. | |

No. 7—Control.
No. 8—Control.

## EXPERIMENTS WITH YOUNG GUINEA-PIGS

Three series of tests were made with young guinea-pigs to see whether there could be produced any indication of sensitization against gelatin. Young pigs, averaging about 300 gm. in weight, were used. The pigs were obtained from three entirely different lots, so as to reduce to a minimum any peculiar idiosyncrasies of certain lots. The sensitizing doses were all given intraperitoneally and varied from 0.005-0.05 gm.: One c c of the dissolved gelatin was made to contain the desired amount for injection. The mode of reinjection varied with the different series. Thus, in Series 1 and 3, the material was reinjected intracardially or intraperitoneally. In Series 2, the reinjection was made intrajugularly.

Tables 2, 3, and 4 show in detail the method employed and the results obtained.

The results in all the experiments with guinea-pigs were quite constant. Only a few of the pigs, in which the second injection was made directly into the jugular vein, was there any evidence whatever of anaphylactic symptoms. Even here the symptoms were so mild as not to indicate a positive reaction, and are to be ascribed, in part, to shock consequent to the anesthetic and the operation exposing the jugular, and in part to the natural primary toxicity of the gelatin. Buglia[29] described a similar series of symptoms in dogs which had received a single intravenous injection of gelatin.

In many cases there was a slight drop in the temperature, but this never exceeded 1.8 C., and was quite transitory, the return to normal being accomplished within 1½ hours, as a maximum.

The coagulation time of the blood, as determined by regular standard methods, showed marked variations. In certain instances an increase of 20-40% in the coagulation time occurred; in other instances there was no change; in still others, an actual decrease. Hence, this must not be considered in reaching conclusions regarding anaphylaxis with gelatin.

TABLE 4

ANAPHYLAXIS EXPERIMENTS WITH GELATIN ON YOUNG GUINEA-PIGS. SERIES 3

| Guinea-pig | Weight, gm. | Sensitizing Injection | | Interval, Days | Second Injection | | Symptoms | Interval, Days | Third Injection | | Symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Amount, gm. | Mode | | Amount, gm. | Mode | | | Amount, gm. | Mode | |
| 1 | 300 | 0.001 | INTRAPERI-TONEAL | 15 | 0.23 | INTRACARDIAL | Negative | | | INTRA-PERI-TONEAL | |
| 2 | 280 | 0.001 | | 15 | 0.20 | | Doubtful | 8 | 0.20 | | Negative |
| 3 | 250 | 0.005 | | 15 | 0.25 | | Negative | 8 | 0.3 | | Negative |
| 4 | 300 | 0.005 | | 15 | 0.25 | | Negative | | | | |
| 5 | 250 | 0.005 | | 15 | 0.20 | | Negative | 8 | 0.3 | Intra-peri-toneal | Negative |
| 6 | 275 | 0.005 | | 15 | 0.20 | | Doubtful | | | | |
| 7 | 300 | 0.25 | intra-car-dial | .. | ...... | | Negative | | | | |
| 8 | 290 | 0.25 | | .. | ...... | | Doubtful | | | | |

No. 7—Control.
No. 8—Control.

Controls were similarly treated throughout, and the symptoms presented were practically identical with those in the animals presumably sensitized.

Only 2 of the pigs tested died—Nos. 1 and 2, Series 1. In neither case were the anatomic findings those recognized as characteristic of death from anaphylactic shock.

In none of the animals subjected to a third injection was there any evidence of a condition of increased refractiveness, simulating antianaphylaxis. The symptoms following the third injection could not be differentiated from those following the second.

Considering all factors involved and the results obtained in the series of 27 guinea-pigs, one must conclude that gelatin is not capable of functioning as an antigen, as expressed by anaphylactic reactions in young guinea-pigs, corroborating the observation of Wells.[21]

## EXPERIMENTS WITH YOUNG DOGS

To determine whether gelatin is capable of sensitizing dogs to subsequent injections of the homologous material, 2 young normal dogs, weighing respectively 3.8 kg. and 4.7 kg. were injected intraperitoneally with gelatin; the one receiving 0.1 gm., the other 0.5 gm. gelatin dissolved in sterile physiologic salt solution so as to make a 5% solution. The dogs showed no reaction whatever to these preliminary injections of gelatin.

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[21] Jour. Am. Med. Assn., 1908, 50, p. 527.

After an interval of 15 days, they received a second injection. The dose on reinjection was 2 gm. of gelatin for the smaller dog, and 3 gm. for the larger. The total volume of material injected was 10 c c and 12 c c, respectively. The second injection was made into the femoral vein.

Blood pressure tests prior to the second injection and subsequent to it showed no fall more marked than that exhibited by a normal dog during an interval of 45 minutes. Necropsy failed to show any of the anatomic findings characteristic of death due to anaphylaxis.[5] No changes were observed in the coagulation time of the blood.

TABLE 5

ANAPHYLAXIS EXPERIMENTS WITH GELATIN ON YOUNG DOGS

| Dog | Weight, gm. | Sensitizing Injection | | Interval, Days | Second Injection | | Results | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Amount, gm. | Mode | | Amount, gm. | Mode | Blood Pressure | Coagulation Period | | General Symptoms | Necropsy |
| | | | | | | | | Pre-injection | Post-injection | | |
| 1 | 3,800 | 0.1 | Intraperitoneal | 15 | 2.0 | Intravenous | The difference in blood pressure before and after injection did not vary 10 mm. of Hg. during an interval of 45 minutes observation | 2'30" | 2'35" | Negative | Negative |
| 2 | 4,700 | 0.5 | Intraperitoneal | 15 | 3.0 | Intravenous | | 3'15" | 3'25" | Negative | Negative |
| 3 | 4,500 | ...... | .. | .. | 3.0 | Intravenous | No change in pressure following injection greater than 10 mm. | 2'40" | 2'50" | Negative | Negative |

These consistently negative results are in accord with those of Aronsen, who attempted to sensitize dogs against gelatin.

## THE PRECIPITIN REACTION

In order to study the precipitin reaction, young normal rabbits were used. Various modes of injection were employed. In one series the intensive method of immunization, first advocated by Fornet and Miller,[22] and later by others[23] was used. This consists of daily intraperitoneal injections of the antigen. In some cases slower methods, consisting of intravenous injections at intervals of about 5 days, were employed. In still other cases, a combination of the two methods previously mentioned were used. Two or three injections were given the rabbit by one of the above methods, and after an interval of 10-12 days the animal was bled and tests made. Following a rest period, which varied from a few days to a month, the animals were reinjected in a similar manner. Many of the rabbits used received from 0.6-12.0 gm. of gelatin during the entire period of treatment. In certain instances this period of treatment was as long as 9 months.

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[22] Ztschr. f. biol. Technik u. Methodik, 1908, 1, p. 201.

[23] Bonhoff and Tszuki: Ztschr. f. Immunitätsf., 1908, 1, p. 201.

The dosage of the gelatin used per injection varied, of course, with the method employed. The details as regards the amounts of gelatin, periods of injections, etc., will be found in Tables 6 and 7. The gelatin was dissolved in sterile physiologic salt in such dilutions as to make the solution 0.5% gelatin. The injections of the gelatin were well tolerated by the rabbits. Only slight loss of weight, which was temporary, and slight stupor were observed.

The blood was obtained in the usual manner by bleeding from the posterior auricular vein, and was allowed to clot spontaneously. The serum was removed and kept on ice until used. Tests were always made within 24 hours after drawing. While precipitins do not disappear rapidly from a serum on standing, the effort was always made to use relatively fresh serum.

TABLE 6

SUMMARY OF INJECTIONS OF RABBITS WITH GELATIN FOR ANTIBODY REACTIONS

| Rabbits | Injections | | | | | No. Tests | Time of Precipitin Tests |
|---|---|---|---|---|---|---|---|
| | Groups of Injections | Total No. Injections | Mode | Total Gelatin, gm. | Interval of Injection | | |
| 1 | 6 | 17 | Intraperitoneal | 12 | July 23, 1916-Dec. 3, 1916 | 6 | Aug. 4, 1916-Dec. 18, 1916 |
| 2 | 5 | 14 | Intraperitoneal | 12 | July 23, 1916-Dec. 1, 1916 | 5 | Aug. 4, 1916-Dec. 9, 1916 |
| 3 | 9 | 25 | Intraperitoneal<br>Intravenous | 9.8<br>5.0 | July 23, 1916-May 1, 1917 | 11 | Aug. 4, 1916-May 15, 1917 |
| 4 | 5 | 12 | Intravenous | 2.0 | Nov. 19, 1916-Mar. 24, 1917 | 4 | Dec. 1, 1916-Apr. 4, 1917 |
| 5 | 2 | 6 | | 1.8 | Aug. 8, 1916-Oct. 1, 1916 | 3 | Aug. 23, 1916-Oct. 12, 1916 |
| 6 | 5 | 10 | | 1.5 | Nov. 19, 1916-Apr. 21, 1917 | 4 | Dec. 3, 1916-May 2, 1917 |
| 7 | 4 | 10 | | 1.6 | Nov. 19, 1916-Aug. 21, 1917 | 2 | Dec. 5, 1916-May 2, 1917 |
| 8 | 1 | 3 | | 0.8 | May 26-29, 1917 | 2 | June 6 and June 9, 1917 |
| 9 | 1 | 3 | | 0.8 | May 26-29, 1917 | 2 | June 6 and June 9, 1917 |
| 10 | 1 | 3 | | 0.8 | May 26-29, 1917 | 2 | June 6 and June 9, 1917 |
| 11 | 1 | 3 | | 0.8 | May 26-29, 1917 | 2 | June 6 and June 9, 1917 |

In the tests, the general procedure was to make various dilutions of the antigen (gelatin) in sterile physiologic salt solution and filter until perfectly free from suspended matter; the filtrate was placed in clean tubes, 1.5 c c in each tube. By means of a capillary pipet, 0.1 c c of the serum was layered beneath the gelatin. In a few instances, the serum was diluted and the gelatin kept constant. Readings were made after 20-30 minutes at room temperature after 2 hours at 37 C., and again after remaining at refrigerator temperature over night.

A total of 11 rabbits were used in the determination of the precipitin reaction, many being tested several times. Tables 6 and 7 show the distribution of the tests on the different animals used, the details of the immunization and the tests.

All tests were made according to this plan—all uniformly negative. As controls, tests were made with serum from rabbits injected with various other proteins, e. g., egg albumin, sheep serum, etc., the customary precipitin reactions here appearing very definitely.

These results indicate that in rabbits gelatin does not possess the property of stimulating the production of specific precipitins when introduced as described.

## COMPLEMENT FIXATION

Since complement fixation is, in general, one of the most delicate tests for the detection of specific antibodies, a series of such tests were made, using serum of the same rabbits as for precipitin reactions. The plan was to make all the various tests at each drawing of the blood. The total number of complement fixation tests was smaller, however. The details follow:

### TABLE 7

The following protocol of a single test will serve to illustrate the details of the precipitin tests:

PRECIPITIN TEST

| Tube | A. Serum Constant, 0.1 c c Gelatin Variable | | | | B. Gelatin Constant, 0.02 gm. Serum Variable | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount of Gelatin gm. | Results | | | Serum Dilution | Results | | |
| | | 30 min. | 2 hrs. | 12 hrs. | | 30 min. | 2 hrs. | 12 hrs. |
| 1 | 1 c c = .05 | 0 | 0 | 0 | 1.4 c c of 1/10 | 0 | 0 | 0 |
| 2 | .04 | 0 | 0 | 0 | 1/20 | 0 | 0 | 0 |
| 3 | .02 | 0 | 0 | 0 | 1/50 | 0 | 0 | 0 |
| 4 | .01 | 0 | 0 | 0 | 1/100 | 0 | 0 | 0 |
| 5 | .005 | 0 | 0 | 0 | 1/300 | 0 | 0 | 0 |
| 6 | .003 | 0 | 0 | 0 | 1/600 | 0 | 0 | 0 |
| 7 | .002 | 0 | 0 | 0 | 1/1,000 | 0 | 0 | 0 |
| 8 | .001 | 0 | 0 | 0 | 1/2,000 | 0 | 0 | 0 |
| 9 | .0005 | 0 | 0 | 0 | 1/5,000 | 0 | 0 | 0 |
| 10 | .05 (no serum) | 0 | 0 | 0 | 1/10 + salt | 0 | 0 | 0 |
| 11 | 1.4 c c salt (control) | 0 | 0 | 0 | No serum, salt + gelatin | 0 | 0 | 0 |

Gelatin is expressed in gm. per tube; total volume, 1.5 c c.

*"Antigenic" Solutions.*—The gelatin was dissolved in physiological salt solution and then standardized. This consisted in determining the normal anticomplementary value of the gelatin, and its nonspecific hemolytic properties. The anticomplementary value as determined a number of times was quite constant. The nonspecific hemolytic action did not manifest itself until quantities much larger than the anticomplementary value were used. In actual tests not more than ⅓ to ½ the anticomplementary value was used.

Hemolytic amboceptor was the serum of rabbits injected with sheep corpuscles. The serum was capable of completely hemolyzing 1 unit volume of erythrocytes in dilutions of 1:3000. In actual testing, 2 such units of amboceptors were used.

A 3% suspension of well washed sheep corpuscles was used.

Fresh guinea-pig serum served as complement; 1½ times the standard unit was used.

The serum from the rabbits was inactivated by heating to 56 C. for 30 minutes.

In the fixation tests, two methods were used. The one most extensively employed was the original method of Bordet in which a constant quantity of the properly standardized antigen is used and varying amounts of serum. The other method, used in a relatively few instances, was that suggested by Kilgore and Myers,[24] and others, in which the amount of antigen is varied, and the serum kept constant.

The antigen, inactivated serum, and complement were mixed in the order named and incubated 1 hour at 37 C., when the hemolytic system was added. The mixture was then incubated 1½ hours at 37 C. Readings were made immediately after incubation and again after remaining in a refrigerator over night. The usual control tests were always made.

A total of 29 tests by the original Bordet method were made using serum from 11 different rabbits. A total of 10 tests were made by the second method.

The following protocols will illustrate the details of the procedure:

TABLE 8

COMPLEMENT FIXATION. GELATIN (ANTIGEN) CONSTANT: SERUM VARIABLE

| Tube | Gelatin | Serum | Guinea-Pig Serum Complement | Salt | | Hemolytic System | | Results | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Amboceptor | Erythrocytes | After 1½ Hrs. Incubation | After 12 Hrs. on Ice |
| 1 | ½ c c = .002 gm. | 0.1 c c | 0.4 c c | To make volume equal 1½ c c | Incubate 1 hr. at 37 C. | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 2 | ½ c c = .002 gm. | 0.05 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 3 | ½ c c = .002 gm. | 0.02 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 4 | ½ c c = .002 gm. | 0.01 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 5 | ½ c c = .002 gm. | 0.005 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 6 | ½ c c = .002 gm. | 0.000 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 7 | 0 | 0.1 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 8 | 0 | 0 | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 9 | 0 | 0 | 0 | | | 0.3 c c | 0.3 c c | No hemolysis | No hemolysis |
| 10 | 0 | 0 | 0.4 c c | | | 0 | 0.3 c c | No hemolysis | No hemolysis |
| 11 | 0 | 0 | 0 | | | 0 | 0.3 c c | No hemolysis | No hemolysis |

Anticomplementary volume of gelatin = 0.006 gm.

The results were constantly negative throughout the entire series. There is no indication of the presence of any substance in the serum of injected animals, which in the presence of "antigen" (gelatin) is capable of binding complement. Thus, as in the case of precipitins, gelatin does not exercise any antigenic functions in the bodies of young rabbits that make themselves evident by the binding of complement.

## THE PRODUCTION OF SPECIFIC PROTEOLYTIC FERMENTS

The introduction of alien proteins into the body of an animal, parenterally, is now generally considered to result in the production of an excess of ferment capable of breaking down and destroying the material introduced. The exact nature of the process, and the character of the ferments are still open to discussion. Whether there is actual production[25] of specific ferments, whether it is a process of mobilization of nonspecific ferments, or whether it is an adsorption[26] of normal antiferments, permitting the action of nonspecific normal ferments, are questions that cannot be considered settled.

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[24] Arch. Int. Med., 1916, 19, p. 293.

According to Vaughan[27] and his school, these ferments are specific and manifest themselves in vivo, with a digestion of the specific material on reinjection—thus explaining anaphylaxis as an intoxication due to toxic protein products. Assuming the truth of this view, it is evident that gelatin did not give any indications of the production of ferments, as shown in the series of anaphylactic tests.

TABLE 9

COMPLEMENT FIXATION. GELATIN (ANTIGEN) VARIABLE: SERUM CONSTANT

| Tube | Gelatin | Serum | Guinea-Pig Serum Complement | Salt | | Hemolytic System | | Results | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Amboceptor | Erythrocytes | After 1½ Hrs. Incubation | After 12 Hrs. on Ice |
| 1 | 0.0012 gm. | 0.1 c c | 0.4 c c | To make volume equal 1½ c c | Incubate 1 hr. at 37 C. | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 2 | 0.0012 gm. | 0.2 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 3 | 0.002 gm. | 0.1 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 4 | 0.002 gm. | 0.2 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 5 | 0.003 gm. | 0.1 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 6 | 0.003 gm. | 0.2 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 7 | 0.003 gm. | 0.0 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 8 | ........... | 0.2 c c | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 9 | ........... | ...... | 0.4 c c | | | 0.3 c c | 0.3 c c | Complete hemolysis | Complete hemolysis |
| 10 | ........... | ...... | 0 | | | 0.3 c c | 0.3 c c | No hemolysis | No hemolysis |
| 11 | ........... | ...... | 0.4 c c | | | 0 | 0.3 c c | No hemolysis | No hemolysis |
| 12 | ........... | ...... | 0 | | | 0 | 0.3 c c | No hemolysis | No hemolysis |

According to Abderhalden, it is possible to demonstrate the presence of these ferments in vitro.

The mixing of a specific antiserum with its antigen results in a cleavage process made manifest by various means, chief of which are changes in the dialyzable character of the antigen, and differences in the way they rotate a beam of polarized light.

Abderhalden reports that he was able to detect such ferments in the serum of dogs, injected with gelatin, as shown by changes in rotation of light. The extent of the change was slight and not strictly specific.

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[25] Abderhalden: Defensive Ferments of the Animal Organism, trans. by J. O. Gavronsky and W. F. Lanchester, 1914.

[26] Jobling and Peterson: Jour. Exper. Med., 1914-16 (V. 19-22).
Bronfenbrenner: Ibid., 1913, 18, p. 221.

[27] Vaughan: Protein Split Products in Relation to Immunity and Disease, 1913.

The same rabbits previously mentioned, and a few new ones not subjected to prolonged treatment, were used to see whether any evidence of such proteolytic ferments could be detected with the gelatin as prepared for these experiments. The rabbits were generally tested within 5 days after the last injection; both the "dialysis" method and the "polariscopic" method were used. The greater number of tests were by the first method.

*A. Dialysis Method. The Substrate.*—The gelatin was dissolved in sterile distilled water. The solution varied in different tests from 1-3%. This material was freshly prepared just before each test and carefully tested for the presence of any diffusible substances normally present.

The Dialyzing Sacs: Two types of dialyzing sacs were used—Schleicher and Shull's parchment sacs, No. 579a, and sacs prepared from celloidin. Parallel tests were carried out with the two kinds of sacs.

All sacs were carefully standardized prior to being used; they were subjected to the following tests.

(a) Impermeability to gelatin solutions of the strength used.

(b) Impermeability to complex proteins, e. g., egg albumin, and rabbit serum.

(c) Permeability to peptones and proteoses as shown by the diffusibility of solutions of Witte's peptone.

*The Serum:* As has been observed by many, and particularly emphasized by Abderhalden,[25] the character of the serum is of primary importance. Great care was exercised therefore in collecting it. Rabbits were bled only after a period of fasting, since it has been shown that serum drawn shortly after meals frequently contains dialyzable products. The blood was drawn from the ear vein, and by using care could be obtained free from visible hemoglobin. Blood tinged with hemoglobin was discarded. No attempt was made to demonstrate hemoglobin spectroscopically, but controls were used to check up any normal dialyzable products. In a few instances the serum obtained was subjected to a predialysis, using as an agent physiologic salt solution, for a period varying from 6-24 hours. Prolonged dialysis was found unsatisfactory since it robbed the serum of any ferment action, as shown by controls. The results obtained with predialyzed serum did not vary much from those obtained when undialyzed serum was used. All serums were used within 4-6 hours after drawing the blood.

*The Test:* This was carried out by placing 1 c c of the gelatin solution and 0.5 c c of the serum to be tested into well cleaned and standardized sacs. The outer surface of the sacs was carefully washed in running distilled water to remove any possible protein contamination, and then placed into vessels containing 10 c c of sterile distilled water. The material within the sac and the water within the vessel were covered with a layer of toluene. The vessels were then covered with close fitting glass covers. The tests were always in duplicate, using both a parchment and a celloidin sac for each test. The sacs were incubated either at room temperature for 24 hours, or at 37 C. for 16 hours. The attempt was made to maintain as uniform conditions as possible. After the period of incubation, 5 c c of the dialysate were removed to a perfectly cleaned tube, and tested for the presence of diffusible proteins as directed by Abderhalden,[25] using ninhydrin throughout. The boiling time was definite and constant, being one minute in all cases.

The depth of color obtained is indicated by the usual "+" signs. Thus "+ + + +" indicates a color of maximum intensity, while "+ + +," "+ +,"

"+," and "O" indicate colors of decreasing intensity to complete absence. Readings of all tests were made 40 minutes after the cessation of boiling, and in good white light.

The following control tests were made and the results compared with those of the test proper. (a) Normal rabbit serum vs. gelatin; (b) anti-ox-serum vs. gelatin; (c) anti-coli-serum vs. gelatin; (d) anti-chondrin serum vs. gelatin; (e) anti-egg albumin serum vs. gelatin, and (f) anti-gelatin serum vs. chondrin.

Table 10 is a composite protocol indicating the character of the tests and the results. The serum of 9 rabbits injected with gelatin was used in 18 tests, 15 of which were with undialyzed serum and 3 with dialyzed.

TABLE 10

PROTEOLYTIC FERMENTS. SERUM NOT PREDIALYZED

| Tube | Substrate | Serum | Results With Rabbit | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | No. 1 | | No. 2 | | No. 5 | |
| | | | 10/14 | 12/11 | 10/14 | 12/11 | 10/15 | 4/16 |
| 1 | 1 c c gelatin 1-5% | 0.5 c c antigelatin | +++ | tr | +++ | ++ | ++ | 0 |
| 2 | 1 c c gelatin 1-5% | 0.5 c c antigelatin | +++ | + | +++ | ++ | +++ | + |
| 3 | 1 c c gelatin | 0.5 c c physiological salt | .. | .. | .. | .. | .. | .. |
| 4 | 1 c c physiological salt | 0.5 c c antigelatin | + | 0 | tr | 0 | + | 0 |
| 5 | 1 c c chondrin | 0.5 c c antigelatin | + | + | + | 0 | + | 0 |
| 6 | 1 c c chondrin | 0.5 c c antigelatin | 0 | tr | + | + | + | 0 |
| 7 | 1 c c gelatin | 0.5 c c antiprecipitin | .. | .. | .. | .. | .. | .. |
| 8 | 1 c c gelatin | 0.5 c c antiprecipitin | .. | .. | .. | .. | .. | .. |
| 9 | 1 c c gelatin | 0.5 c c anticoli | .. | .. | .. | .. | .. | .. |
| 10 | 1 c c gelatin | 0.5 c c anticoli | .. | .. | .. | .. | .. | .. |
| 11 | 1 c c gelatin | 0.5 c c normal | .. | .. | .. | .. | .. | .. |
| 12 | 1 c c gelatin | 0.5 c c normal | .. | .. | .. | .. | .. | .. |
| 13 | 1 c c physiological salt | 0.5 c c normal | .. | .. | .. | .. | .. | .. |
| 14 | 1 c c physiological salt | 0.5 c c anticoli | .. | .. | .. | .. | .. | .. |
| 15 | 1 c c physiological salt | 0.5 c c antiprecipitin | .. | .. | .. | .. | .. | .. |
| 16 | 1 c c gelatin | 0.5 c c chondrin | .. | .. | .. | .. | .. | .. |

Proteolytic Ferments. Serum Predialyzed

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 4/16 | 4/17 |
| 1 | 1 c c gelatin 1-5% | 0.5 c c antigelatin | .. | .. | .. | .. | +++ | +++ |
| 2 | 1 c c gelatin | 0.5 c c antigelatin | .. | .. | .. | .. | +++ | +++ |
| 3 | 1 c c salt solution | 0.5 c c antigelatin | .. | .. | .. | .. | + | + |
| 4 | 1 c c chondrin | 0.5 c c antigelatin | .. | .. | .. | .. | ++ | ++ |
| 5 | 1 c c chondrin | 0.5 c c antigelatin | .. | .. | .. | .. | ++ | ++ |

Tubes 7 and 8 represent a precipitin serum. In a part of the tests this was an antisheep serum, active against its specific antigen in a dilution of 1:10,000; in another group it was an egg albumin serum with a specific activity of 1:15,000.

Tubes 9 and 10 represent an anti-coli-serum which agglutinated its homologous antigen in dilutions ranging from 1:1000 to 1:1500.

The results tabulated seem to indicate that the injection of the rabbits with gelatin has resulted in the increased presence of proteolytic ferments. The results, however, are neither uniform nor specific. Thus Rabbits 1, 3, and 5 show periods when these ferments are quite marked, while at other times they are practically lacking. The lack of specificity is observed in the presence of dialyzable material in Tubes 7 and 8, 9 and 10, and 16. At most, the results would seem to indicate a quantitative specificity and this is neither marked nor constant. These results agree in the main with those of Elsesser[4] as regards the specificity of proteolytic ferments as revealed by the dialyzing technic of Abderhalden.

Serum, predialyzed, did not yield any more conclusive results than undialyzed serum.

No difference could be noted in the efficiency of the celloidin and parchment sacs, when properly standardized. Sac 1 in duplicate is a Schleicher and Shull 579a, and 2 a celloidin sac.

*B. Optical Method*—Since Abderhalden's report on an antigelatin ferment is based on the polariscopic method, 2 rabbits were injected intravenously with gelatin and tested according to this method.

TABLE 10—*Continued*

PROTEOLYTIC FERMENTS. SERUM NOT PREDIALYZED

| Results With Rabbit | | | | | | | | | Controls | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. 7 | | No. 9 | No. 10 | No. 11 | No. 12 | No. 3 | | | | | | | | | |
| 12/11 | 4/16 | 6/13 | 6/13 | 6/13 | 6/13 | 10/14 | 12/11 | 14-16 | 10/14 | 10/15 | 12/11 | 4/16 | 6/13 | 6/17 | 4/16 |
| +++ | +++ | +++ | 0 | ++ | + | +++ | ++ | 0 | | | | | | | |
| +++ | +++ | ++ | + | + | + | +++ | +++ | + | | | | | | | |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | 0 | 0 | + | + | + | 0 |
| + | + | + | + | 0 | tr | 0 | tr | 0 | | | | | | | |
| ++ | + | ++ | + | + | ++ | ++ | +++ | + | | | | | | | |
| + | + | + | + | tr | tr | ++ | ++ | 0 | | | | | | | |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | tr | + | + | +++ | + | + | ++ |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | tr | + | + | ++ | 0 | + | ++ |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | + | ++ | + | + | 0 | 0 | tr |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | + | ++ | + | + | tr | 0 | + |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | + | 0 | + | tr | 0 | + |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | + | tr | 0 | 0 | tr | + |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | tr | tr | 0 | 0 | tr | tr | tr |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | 0 | tr | 0 | 0 | + | + |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | 0 | 0 | 0 | 0 | 0 | tr | 0 |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | +++ | ++ | ++ | ++ |

Proteolytic Ferments. Serum Predialyzed

| | | | | 6/17 | | | 4/16 | 4/17 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| .. | .. | ++ | ++ | .. | .. | .. | +++ | +++ | | | | | | | |
| .. | .. | ++ | + | .. | .. | .. | +++ | +++ | | | | | | | |
| .. | .. | tr | + | .. | .. | .. | + | 0 | | | | | | | |
| .. | . | ++ | tr | .. | .. | .. | ++ | tr | | | | | | | |
| .. | .. | + | 0 | .. | .. | .. | + | tr | | | | | | | |

These rabbits received a total of 1.0 gm. of gelatin in three groups of injections between June 25 and Aug. 9, 1917. One rabbit received a total of 7 injections; the other received 6 injections.

The method outlined by Abderhalden was followed and the tests made 5 days subsequent to the last injection. Fresh serum, drawn only a few hours prior to testing was used. Serum from a normal rabbit was used as a control.

The basis for recording the results as negative is the fact that the variations in the degree of rotation of the polarized light were no greater when using the serums from treated animals than when the normal serum was used. Neither was the reaction specific, as shown by similar results being obtained when testing the serums with the homologous substrate, the heterologous substrate, or the salt solution control.

While the number of tests carried out was very limited, the general results gave no indications that a specific proteolytic ferment had been produced by repeated injections of gelatin into rabbits.

### MEIOSTAGMIN REACTION

Ascoli and Izar attempted to show that the production of specific antibodies, as a result of injections, could be made evident by certain physicochemical methods dependent on the changes in the viscosity resulting from the interaction between the serum and the antigen. Many others since that time have reported results with this method but with lack of uniformity. Izar[24] in a later publication reports positive results when serum from rabbits injected with gelatin was tested in this way. No attempt is made to establish the efficiency of the meiostagmin reaction in this work, but simply to duplicate the work of Izar with the hope that it might contribute something to the question of the antigenic properties of gelatin.

A series of rabbits consisting of Nos. 2, 3, 5, and 7 used in other tests, with 2 rabbits injected with chondrin and 2 normal rabbits, were used.

TABLE 11

POLARISCOPIC METHOD EMPLOYED

| Test | Rabbit | Amount Gelatin Injected Intravenously | Interval of Injections | Total No. Injections | Substrate | Amount of Serum | Results |
|---|---|---|---|---|---|---|---|
| 1 | (a) | 1 gram | June 25 to Aug. 9, 1917 | 7 | Gelatin 1% | 1½ c c | Negative |
|  | (a) |  |  |  | Albumin 1% | 1½ c c | Negative |
|  | (a) |  |  |  | Salt solution | 1½ c c | Negative |
| 2 | (b) | 1 gram |  | 6 | Gelatin 1% | 1½ c c | Negative |
|  | (b) |  |  |  | Albumin 1% | 1½ c c | Negative |
|  | (b) |  |  |  | Salt solution | 1½ c c | Negative |
| 3 | (c) | Normal rabbit |  | 0 | Gelatin 1% | 1½ c c | Negative |
|  | (c) |  |  |  | Albumin 1% | 1½ c c | Negative |
|  | (c) |  |  |  | Salt solution | 1½ c c | Negative |

One series of experiments was carried out following the general method discussed by Ascoli and Izar; the other series by a slightly different method described by Izar. The principles involved in both are alike, being simply a difference in details.

The indication of a positive reaction is a decrease in the surface tension of the solution with an increase in the number of drops in a unit volume of the antigen-antibody mixture. Also a difference in the rate of delivery when the mixture is discharged from a special capillary pipet. In this work a Traube stalagmometer was used.

*Technic. First Method.*—The instrument was carefully cleaned with water followed by several washings with absolute alcohol and ether.

This process of cleaning was repeated after each test. The instrument was finally rinsed in the fluid to be tested. To insure uniformity of temperature, which is an important factor in viscosity tests, the instruments were placed inside an incubator, and tests conducted in the closed chamber. The variations in temperature did not exceed 2½ C. The incubator, a large heavy one, was placed on a cement floor in a basement to eliminate any confusing vibratory factors.

The serum was obtained 5 days subsequent to the last injection and used within 10 hours after drawing the blood. The serum was diluted with equal volumes of physiologic salt solution. The gelatin was similarly diluted 1 : 1000. In the test, 9 c c of the diluted serum was thoroughly mixed with 1 c c of the diluted gelatin, and the number of drops per unit volume immediately determined. The mixture was then incubated 1 hour at 37 C. and the

TABLE 12

SERIES A. MEIOSTAGMIN REACTION WITH GELATIN VS. RABBIT SERUM

| No. Rabbit | Serum | Antigen | Results. No. Drops per Unit Volume | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Preincubation | | | Postincubation, 1 Hour at 37 C. | | |
| 2 | 9 c c–1:1 | 1 c c gelatin 1:100 | 64.1 | 64.0 | 64.2 | 64.3 | 64.2 | 64.0 |
| 2 | 9 c c–1:1 | 1 c c chondrin 1:100 | 63.4 | 63.6 | 63.4 | 63.5 | 64.1 | 63.8 |
| 3 | 9 c c–1:1 | 1 c c gelatin 1:100 | 63.7 | 63.5 | 63.8 | 62.8 | 63.4 | 62.9 |
| 3 | 9 c c–1:1 | 1 c c chondrin 1:100 | 62.8 | 62.7 | 63.0 | 62.9 | 63.0 | 63.1 |
| 5 | 9 c c–1:1 | 1 c c gelatin 1:100 | 62.9 | 63.5 | 63.2 | 63.1 | 63.6 | 63.2 |
| 5 | 9 c c–1:1 | 1 c c chondrin 1:100 | 63.0 | 63.1 | 62.8 | 63.4 | 63.2 | 63.0 |
| 7 | 9 c c–1:1 | 1 c c gelatin 1:100 | 64.3 | 64.1 | 64.4 | 64.0 | 64.2 | 64.1 |
| 7 | 9 c c–1:1 | 1 c c chondrin 1:100 | 63.6 | 63.8 | 63.6 | 63.8 | 64.0 | 64.0 |
| Normal | 9 c c–1:1 | 1 c c gelatin 1:100 | 62.4 | 62.9 | 62.8 | 62.3 | 62.7 | 62.9 |
| Normal | 9 c c–1:1 | 1 c c gelatin 1:100 | 62.8 | 62.7 | 62.9 | 63.1 | 62.6 | 63.0 |

TABLE 13

MEIOSTAGMIN REACTIONS

| No. Test | Nature of Mixture | Results—Rabbit No. 1 | | | | Results—Rabbit No. 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Time of Discharge | | No. Drops | | Time of Discharge | | No. Drops | |
| | | Pre-incubation | Post-incubation | Pre-incubation | Post-incubation | Pre-incubation | Post-incubation | Pre-incubation | Post-incubation |
| a | 4.5 c c immunized rabbit serum+5.5 c c of 0.85% NaCl | 3′45″<br>3′50″<br>3′50″ | 3′50″<br>3′40″<br>3′45″ | 64.1<br>63.6<br>64.0 | 65.2<br>54.2<br>64.0 | 3′30″<br>3′40″<br>3′40″ | 3′25″<br>3′30″<br>3′25″ | 63.1<br>63.4<br>63.2 | 63.0<br>63.1<br>63.1 |
| b | 4.5 c c immunized rabbit serum+4.5 c c NaCl+ 1 c c 1% gelatin sol. | 4′25″<br>4′25″<br>4′25″ | 4′10″<br>4′20″<br>4′20″ | 63.4<br>63.6<br>63.4 | 63.1<br>63.2<br>63.6 | 3′40″<br>3′40″<br>3′35″ | 3′35″<br>3′40″<br>3′35″ | 63.2<br>63.1<br>63.1 | 64.3<br>63.4<br>63.1 |
| c | 1 c c immunized rabbit serum+9 c c of 0.85% of NaCl | 3′00″<br>3′00″<br>3′05″ | 3′00″<br>3′00″<br>3′00″ | 60.1<br>60.1<br>60.3 | 61.0<br>60.2<br>60.3 | 3′05″<br>3′05″<br>3′10″ | 3′05″<br>3′05″<br>3′05″ | 60.1<br>60.0<br>60.9 | 60.0<br>60.5<br>60.3 |
| d | 1 c c immunized rabbit serum+8 c c NaCl+1 c c of 1% gelatin solution | 3′05″<br>3′10″<br>3′10″ | 3′20″<br>3′20″<br>3′20″ | 60.9<br>61.0<br>61.1 | 61.5<br>61.2<br>61.4 | 3′15″<br>3′20″<br>3′15″ | 3′10″<br>3′15″<br>3′15″ | 60.0<br>60.5<br>60.1 | 61.0<br>60.2<br>60.1 |
| e | 9 c c of 0.85% NaCl+1 c c of 1% gelatin solution | 3′10″<br>3′15″<br>3′10″ | 3′20″<br>3′25″<br>3′15″ | 62.1<br>61.5<br>62.0 | 62.2<br>62.0<br>62.1 | | | | |
| f | 4.5 c c normal rabbit serum+4.5 c c NaCl+ 1 c c gelatin solution | 3′55″<br>4′20″<br>4′15″ | 4′10″<br>3′55″<br>3′50″ | 63.2<br>63.2<br>63.1 | 63.0<br>63.4<br>63.2 | | | | |
| g | 1 c c normal rabbit serum+8 c c of 0.85% NaCl+1 c c gelatin sol. | 3′05″<br>3′05″<br>3′10″ | 3′10″<br>3′05″<br>3′10″ | 60.1<br>60.0<br>60.5 | 60.3<br>60.2<br>60.1 | | | | |

number of drops per unit volume again determined. Serum from normal rabbits and from rabbits injected with chondrin was similarly tested as controls. Table 12 shows the results obtained by this method.

*Technic—Second Method.*—Two rabbits were used in this series. They received a total of 0.8 gm. of gelatin intravenously on 3 successive days. The test was made on the 7th day after the last injection. The technique was that of Izar,[24] in his work on gelatin. In this test not only the number of drops per unit volume was determined, but also the rate of discharge. Incubation time was 1 hour at 37 C.

As a result of their work, Ascoli and Izar came to the conclusion that only differences greater than 3 drops, per unit volume, between the preincubation and the postincubation tests constitute a positive result. Taking this as a standard it is evident that in neither of the series here reported is there any indication of a positive reaction. The maximum difference is less than 1.5 drops and even this is not constant.

Thus the results are not in accord with those of Izar,[24] but seem to show that gelatin injected into rabbits does not stimulate the production of antibodies, as shown by changes in viscosity of such a gelatin-antiserum mixture.

## SUMMARY

A study of the results of anaphylactic, precipitin, complement-binding, and meiostagmin reactions, shows that all were constantly negative, with gelatin as an antigen, in rabbits, guinea-pigs, and dogs, the recipients of the injections.

The results when the serums of such animals were tested for the presence of increased proteolytic action are somewhat variable and indefinite. There are some indications that there had been an increase in the ferments active against gelatin, but this is not constant nor marked. The action is not strictly specific since considerable digestion takes place with other substrates. At most, one can say it may be quantitatively specific.

Gelatin as prepared and used in this series of experiments has only a slight toxicity for guinea-pigs, dogs, and rabbits. Such animals tolerate relatively large doses, even when the injections are made intravenously. Neither is there any evidence of any cumulative action of the toxicity of the gelatin, since many of the animals received large quantities over long intervals of time.

No attempt has been made to determine what might be responsible for this evident lack of antigenic property exhibited by gelatin. Whether this is due to the lack of amino-acids containing aromatic radicals, to peculiar molecular characteristics, or to some other factor, is not known. The fact established is, that in the types of animals used and with the tests employed, no antibodies could be detected as the result of antigenic action of gelatin.